Experimental evolution under cell envelope stress in nisin producing *lactococci*

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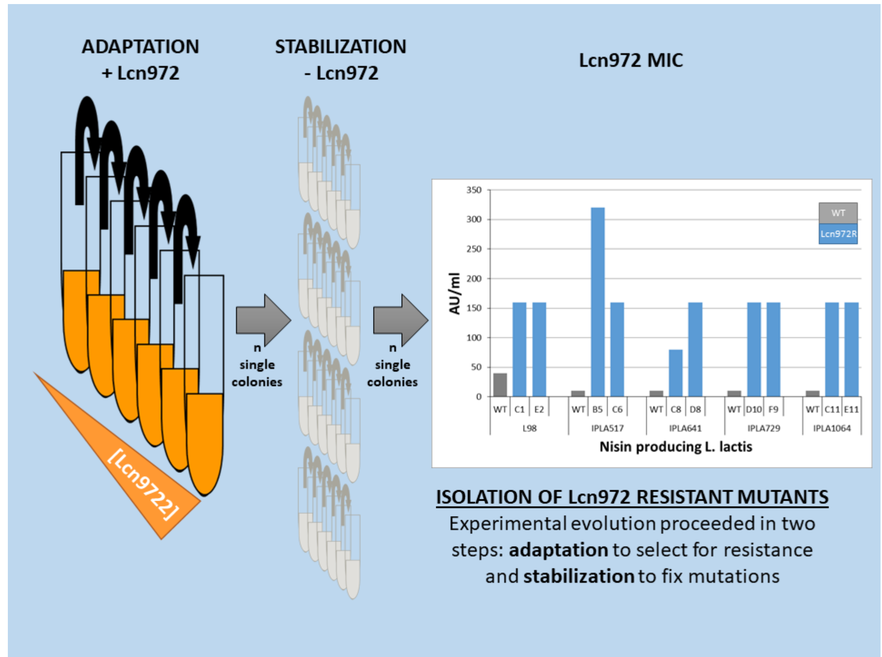
BACKGROUND

Lactococcus lactis is widely used as starter in cheese fermentations and nisin producers are often included in the formulation of **protective cultures** for enhancing food safety. However, developing novel starter blends is hindered by the relatively small biodiversity within available commercial strains.

How can we diversify industrial nisin producing *L. lactis* to develop new protective cultures*?*

Apply **adaptive evolution** under cell envelope stress (AE-CES), using as stressor the bacteriocin **Lcn972** that inhibits cell wall biosynthesis in *Lactococci*.



ISOLATION OF Lcn972 RESISTANT MUTANTS

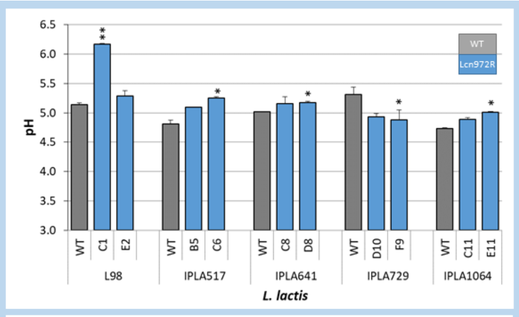
Experimental evolution proceeded in two steps: **adaptation** to select for resistance and **stabilization** to fix mutations

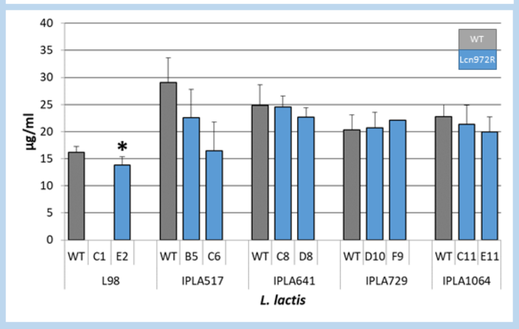
Lcn972R nisin producers do…

… retain growth and nisin production in milk

… change surface properties and some become phage sensitive

… show cross-resistance to other cell wall antimicrobials





MILK ACIDIFICATION

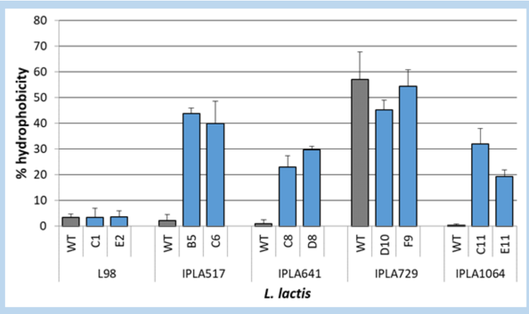
Pasteurized milk incoculated at 3% and incubated for 6 h at 30 °C.

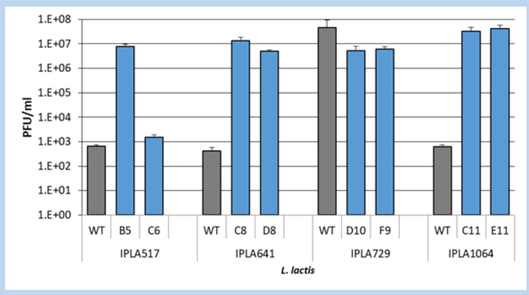
Loss of the lactose plasmid may occur

See mutant *L. lactis* L98_C1 does not growth in milk or produced nisin in milk

NISIN PRODUCTION IN MILK

Pasteurized milk incoculated at 3% and incubated for 6 h at 30 °C.





SURFACE HYDROPHOBICITY

Determined by the MATS method by cell migration to hexadecane.

Loss of an anti-phage plasmid may occur,

See mutants from *L. lactis* IPLA517, IPLA641 and IPLA1064. They become hydrophobic and sensitive to a C2-like phage

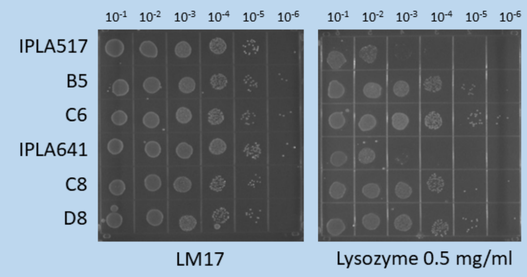
PHAGE SUSCEPTIBLITY

Phage CHPC1180 titers were determined by the double layer agar method.

L. lactis	Bacitracin (1 µg/ml)	Lysozyme (0.5 mg/ml)	Penicillin G (0.1 µg/ml)	Vancomycin (0.4 µg/ml)
L98-C1	=	E	E	S
L98-E2	=	E	E	S
IPLA517-B5	=	R	S	=
IPLA517-C6	=	R	S	R
IPLA641-C8	S	R	S	R
IPLA641-D8	S	R	S	R
IPLA729-D10	S	R	=	=
IPLA729-F9	=	R	=	ND
IPLA1064-C11	S	R	S	R
IPLA1064-E11	=	=	=	R

CROSS-RESISTANCE

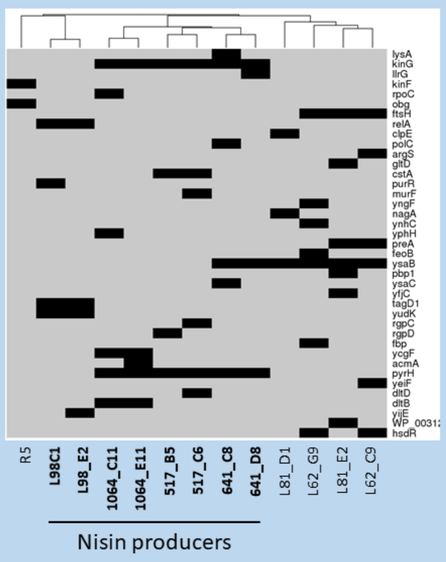
Determined by spotting decimal dilutions of cell suspensions on plates with the selected antimicrobial.
See example below.

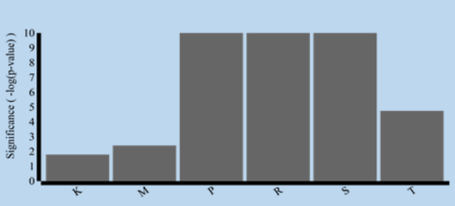


… display non-synonymous mutations

NON-SYNONYMOUS MUTATIONS

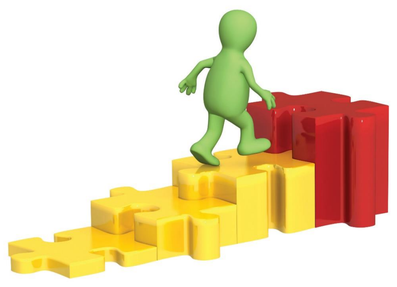
Detection of point mutations was performed with CLC Genomics workbench 10.1.1 and overrepresented COG categories with GSEA_pro.





COG categories
K: Transcription
M: Cell wall/membrane/envelope biogenesis
P: Inorganic ion transport and metabolism
R: General function prediction only
S: Function unknown
T: Signal transduction mechanisms

Lcn972R mutants clustered together according to the parental strain



CONCLUSIONS

- AE-CES introduces phenotypic and genetic diversity in *L. lactis.*
- Evolved strains retain main technological traits
- Strain background influences the outcome of AE-CES
- Cross-resistance to other antimicrobials should be studied further
- Plasmid loss is the main disadvantage of AE-CES

MORE INFO in: López-González *et al.* 2018. Frontiers in Microbiology 9:2654.
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